

## INTERACTION OF DRAXIN AND $\gamma$ -NETRINS

### CROSS REFERENCE TO RELATED APPLICATION

[0001] This application is a divisional of U.S. Ser. No. 15/113,878 filed Jul. 25, 2016, which is a 35 U.S.C. 371 National Phase Entry Application from PCT/EP2015/051088, filed Jan. 21, 2015, which claims the benefit of U.S. Patent Application No. 62/049,643 filed on Sep. 12, 2014 and European Patent Application No. 14152341.5 filed Jan. 23, 2014, the disclosure of which are incorporated by reference in their entirety.

### FIELD OF THE INVENTION

[0002] This invention relates to extracellular protein-protein interactions and their possible therapeutic uses. More particularly, this invention describes the interaction between Draxin, particularly fragments binding to  $\gamma$ -Netrins comprising SEQ ID NO.:1, 2 or 3, and variants thereof, with  $\gamma$ -Netrins, and the use of this interaction to disrupt  $\gamma$ -Netrin/Netrin receptor interactions. The invention also relates to diagnostic and/or therapeutic uses of Draxin or fragments or variants thereof, as well as to an antibody against Draxin inhibiting binding of Draxin to  $\gamma$ -Netrins. Further, the invention relates to fragments of  $\gamma$ -Netrins, in particular Draxin-binding Netrin1-fragments comprising SEQ ID NO.: 51 and variants thereof, as well as to an antibody against  $\gamma$ -Netrins inhibiting binding of  $\gamma$ -Netrins to Netrin receptors.

### SEQUENCE LISTING

[0003] The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Jan. 16, 2018, filed in the parent application Ser. No. 15/113,878 is named 57261 PUSWO\_ST25.txt and is 87,752 bytes in size.

### BACKGROUND OF THE INVENTION

[0004] The laminin-related netrin protein family in humans comprises 5 members. Three of them, Netrin1, Netrin3, and Netrin4 are secreted proteins. NetrinG1 and NetrinG2 instead are linked to the cell surface by a GPI anchor. The LamNT domain and the EGF domains of Netrin1 and Netrin3 are derived from the  $\gamma$  chain of Laminin1. In the context of the present invention, these Netrins are thus referred to as " $\gamma$ -Netrins". In contrast, the corresponding domains of Netrin4, NetrinG1 and NetrinG2 are homologous to the domains present in the  $\beta$  chain of Laminin1 (Moore et al., 2007).

[0005] Netrin1 is a diffusible, laminin-related protein identified as neuronal guidance cue during development of the nervous system. Netrin1 mediates its biological effects through binding to receptors, which belong to the so-called dependence receptors, e.g. deleted in colorectal cancer (DCC) and uncoordinated-5-homolog (UNC5H). Recently, it has been found that Netrin1 is expressed outside the nervous system and contributes to the patterning of developing epithelial tissues such as mammary gland, pancreas, and lung by regulating diverse processes including adhesion, motility, proliferation, and differentiation of cells.

[0006] Numerous tumors have been described to express cell surface receptors belonging to the DCC- and UNC5-family. These receptors are binding to the secreted ligand

Netrin1 in the extracellular space and serve, in addition to their well-established neurodevelopmental function, as dependence receptors in cancers (Castets et al., 2012; Mehlen et al., 2011). In tumors they can regulate tumor cell survival in a Netrin1 dependent manner. Netrin1 itself is known to be upregulated by many tumor types and has been suggested to act as an oncogene (Arakawa, 2004; Fitamant et al., 2008). If Netrin1 is not bound to dependence receptors of the DCC- and UNC5-family, the receptors cannot form dimers or multimers, which in turn triggers the activation of a pro-apoptotic pathway.

[0007] In several studies, decoy Netrin receptor fragments have been used to disrupt Netrin/Netrin receptor interactions in order to induce pro-apoptotic signaling. For example, such receptor fragments have been used in cancer cell lines (Delloye-Bourgeois et al., 2009; Fitamant et al., 2008) and in animal models (Fitamant et al., 2008; Paradisi et al., 2013; Paradisi et al., 2009) to induce cancer cell death. However, using a fragment of a Netrin receptor causes interference at a relatively late stage of the signaling cascade, namely just before dimerization of the receptor. Moreover, even when using high concentrations of these decoy receptors, a residual binding of Netrin to the full length receptor cannot be prevented.

[0008] It was thus an object of the invention to provide compounds that can be used for interfering with the binding of  $\gamma$ -Netrins, in particular Netrin1 to at least one of its receptors, which at least partially overcome the disadvantages of the prior art.

[0009] Draxin is a secreted protein described to be involved in axon guidance decisions (Islam et al., 2009). In contrast to Netrins 1-3, which are present in vertebrates and invertebrates, Draxin can only be found in vertebrate genomes. The amino acid sequence of human Draxin is shown as SEQ ID NO.: 4. In zebrafish, there exist two Draxin isoforms (DraxinA and DraxinB); their amino acid sequences are represented by SEQ ID NO.: 5 and SEQ ID NO.: 6.

[0010] By using an extracellular protein-protein interaction screen assay (AVEXIS), the present inventors identified Draxin as a novel direct binding partner for Netrin1. Furthermore, by using an AVEXIS based competition assay, the inventors were able to show that Draxin or Draxin protein fragments can compete with Netrin receptors for binding to Netrin1.

[0011] The present invention therefore provides specific peptides binding to  $\gamma$ -Netrins (" $\gamma$ -Netrin-binding peptides"), particularly to Netrin1, as well as antibodies directed against  $\gamma$ -Netrins (" $\gamma$ -Netrin-binding antibodies"), particularly against Netrin1, which may be used for interfering with  $\gamma$ -Netrin/Netrin receptor binding, in particular Netrin1/Netrin receptor binding. The invention further provides Draxin-binding peptides as well as Draxin-binding antibodies inhibiting binding of Draxin to  $\gamma$ -Netrins, in particular to Netrin1.

### DETAILED DESCRIPTION OF THE INVENTION

[0012] In one aspect, the present invention relates to peptides which bind to at least one  $\gamma$ -Netrin, with a high specificity and a high affinity. Importantly, the affinity of Draxin to the  $\gamma$ -Netrins, in particular to Netrin1, is significantly higher than the affinity of  $\gamma$ -Netrins, in particular of Netrin1 to Netrin receptors.